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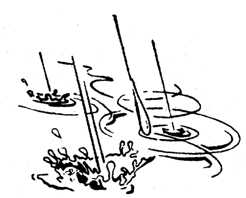
DISCUSSION . . .

Transformations of Carbon by Microorganisms



Dr. Nickerson's excellent review can be related to waste disposal in a direct manner, for he discusses the fundamental biochemical reactions that are carried out by pure cultures of microorganisms in the oxidation of individual chemical compounds. In waste disposal practice complex mixtures of chemical compounds are oxidized by mixed cultures. Let us attempt to see where the theory and practice coincide.

Oxidation of Carbon Compounds

In considering the oxidation of compounds containing carbon, hydrogen, and oxygen, we shall divide them into three classes—carbocyclic compounds, detergents and related compounds, and resistant small molecules.

Carbocyclic Compounds. The fact that phenol, cresol, and related carbocyclic compounds have long been known to undergo rapid biochemical oxidation in trickling filters and activated sludge systems comes as a shock to the bacteriologist who thinks of such compounds only in relation to the "phenol coefficient." Nevertheless, pioneer studies demonstrated the oxidation of phenolic compounds in municipal treatment plants in England before 1900. Similar and quite detailed studies were carried out at Milwaukee, at Chicago, and recently at Gary, Ind., all demonstrating that such wastes are readily oxidized in conjunction with sewage in activated sludge plants. The first industrial waste disposal plant designed to oxidize phenolic wastes was that of the Dow Chemical Co. at Midland, Mich. (*6*). Recent installations, patterned after the Midland studies, are in operation at Sarnia, Ont., and there are doubtless a number of which I am not aware. A noteworthy feature is that chemical-grade phenol is added to such units when the plant is shut down, thus keeping the culture happy and well fed (*3, 11*).

An important feature of the biologica oxidation of phenols is that these compounds are evident to taste in remarkably low concentrations, and the taste threshold of their derivatives produced by chlorination of water is yet lower. Therefore, they must be essentially removed by biochemical oxidation from water that might later be used for a city supply.

The terpenes and rosin waste from wood naval stores production is a related waste that has been oxidized successfully in pilot plant studies (*7*). It is almost certain that these can be treated when the need arises.

Paper mill wastes, consisting of lignin derivatives, pentose, and hexose sugars were long considered to be resistant to biochemical oxidation. The solution to this problem rested on the demonstration by various workers, notably Sawyer and his students (*7, 8*), that nitrogen and phosphorus must be supplied to meet the nutritional requirements of the organisms. High rates of oxidation have been obtained in pilot plant studies and at least one commercial installation is in operation at the West Virginia Pulp and Paper Co., Covington, Va. (*12*).

Detergents and Related Compounds. These important compounds that have the common property of being surface active or of containing a polar and a nonpolar portion within the molecule are mentioned as a class, but it is essential to emphasize that they do not constitute a group of compounds that have a comparable ease of oxidation. They differ widely in susceptibility of oxidation because they differ in chemical nature. This point has been made before, especially by Bogan and Sawyer (*2*). Their work and that of Mills and Stack (*13, 14*) has shown that synthetic organic chemicals can be roughly classified as follows:

Alkyl sulfates Fatty acid derivatives	Readily Oxidized
Alkylaryl sulfonates Alkylphenoxy sulfonates Polyethoxy fatty acid esters Ethers and secondary amines Acrylonitrile	Oxidized with Difficulty
Heterocyclic ring structures Polyglycol ethers	Resistant to Oxidation

Oxidation of Synthetic Organic Compounds

Branching of the alkyl group, not indicated in the above listing, has been proved to reduce markedly the susceptibility of alkyl groups to aerobic assimilation (*2*).

Most of these compounds are detergents; they occur in the wastes from the textile and chemical industries and from the use of these compounds in industry and in the home. They and related synthetic compounds yet to come into large scale use will furnish a continuing challenge to the sanitary engineer, one in which he can draw upon the results of basic investigators of bacterial metabolism to great advantage.

A noteworthy example is the high molecular weight polyethylene glycols, which contain the repeating unit, $(—CH_2—CH_2—O—)_n$, and are thereby polymeric ethers. As yet no one has demonstrated the necessary extracellular enzyme that must be secreted before these compounds can be broken down sufficiently to enter the cell and be metabolized aerobically.

Resistant Small Molecules. Almost all of the compounds containing 2–5 carbon atoms are metabolites in the cell or contain groups readily oxidized to cell intermediates. Nickerson has indicated these relationships very clearly. There are also several noteworthy exceptions, important in industrial waste practice, to which I wish to call attention. Formaldehyde is a potent bactericide, yet Dickerson and associates have successfully treated it by aerobic oxidation (*4*). Pentaerythritol, $C(CH_2OH)_4$, is also oxidized in the same treatment plant. This compound shows no B.O.D. by the usual technique and also passes through animals without toxicity or chemical change. The presence of readily available carbon compounds was shown to assist the oxidation of pentaerythritol. The development of a culture which oxidizes these compounds was a relatively slow and difficult job; acclimatization of the culture to the unusual compound was shown to be important both here and in the laboratory studies of Mills and Stack (*13, 14*).

Common Pathways of Oxidation

The common pathways of oxidation were clearly shown by Nickerson. The compounds in which carbon atoms are combined with oxygen (carbohydrates) or nitrogen (proteins and amino acids) as well as the simple aliphatic residues (from fats and amino acids) readily enter the metabolic pool of the organism and are used for its own vital processes. Many of the enzyme systems depicted do not occur in every microorganism. It is for this reason we find a mixed culture in an activated sludge tank—one that changes in composition with the changing constituents of the waste. Several years ago it was shown that the rate of oxidation of lactose and of casein by an aerated culture developed on milk waste is strictly additive (*9*). This result can best be interpreted as indicating that one group of organisms oxidizes lactose and another group oxidizes casein. Isolation of organisms which grow on one constituent but do not attack the other seemed to confirm this conclusion. Therefore it must be remembered that, although these probably are common pathways in metabolism, they are not all possessed by one organism. This idea is implicit in Nickerson's paper, wherein he speaks of many special transformations carried out by particular organisms.

Cell Synthesis

Finally let us consider cell synthesis—the primary carbon transformation of all microorganisms. In recent years there have been a number of studies of the yield of cell material in aerobic treatment systems (*5, 10, 16*). During the rapid growth phase slightly over 50% of the carbon utilized is transformed to cell tissue; the remaining half is oxidized to obtain energy.

Weston and Eckenfelder have recently concluded that a far higher efficiency of cell synthesis can be calculated from thermodynamic data than this observed value (*16*). It would appear that the cells do not exhibit the thermodynamic efficiency predicted; further study will be required to explain this discrepancy between theory and observation. In such studies care must be taken to prove that the composition of the cells producr does not change—that there is a thermodynamically steady state. Recent evidence that activated sludge can store carbohydrates in an amount equal to about half its original weight indicates that the composition of the cells may vary rapidly (*15*). In such cases the theoretical calculations must be different for short time experiments from those used in developing the equations for a balanced-growth process producing cells of constant composition.

These ideas of the efficiency of cell synthesis and the subsequent endogenous oxidation are of more than theoretical value. They account for the constancy of operation of the mixed culture in aerobic oxidation systems. The concept can be stated as an illustration of the biological principle of selection of species: The organisms that produce the greatest yield of their own cell tissue will thereby prevail in a mixed culture.

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SAM R. HOOVER
Eastern Regional Research Laboratory, Philadelphia, 18, Pa.